

The following listing of claims will replace all prior versions, and listings, of claims in this application.

Listing of the Claims:

Claims 1-21 (Cancelled).

22. (Original) An isolated polypeptide comprising SEQ ID NO:2.

Claims 23-25 (Cancelled).

26. (Original) An G1cNAc-phosphotransferase comprising an α subunit, a β subunit and a site-specific proteolytic cleavage site interposed between said α and β subunits, wherein said site-specific proteolytic cleavage site is not endogenous to G1cNAc-phosphotransferase.

Claim 27 (Cancelled).

28. (Currently Amended) The G1cNAc-phosphotransferase of Claim 26, wherein said α subunit is encoded by nucleotides 165 to 2948 of SEQ ID NO:3, or a sequence that hybridizes under stringent conditions to the complement of nucleotides 165 to 2948 of SEQ ID NO:3, wherein said stringent conditions comprise hybridization in 50% formamide, 1M NaCl, 1% SDS at 37°C and washing in 0.1 X SSC at 60 to 65°C.

29. (Currently Amended) The G1cNAc-phosphotransferase of Claim 26, wherein said β -subunit is encoded by nucleotides 2949 to 3932 of SEQ ID NO:3, or a sequence that hybridizes under stringent conditions to the complement of nucleotides 2949 to 3932 of SEQ ID NO:3, wherein said stringent conditions comprise hybridization in 50% formamide, 1M NaCl, 1% SDS at 37°C and washing in 0.1 X SSC at 60 to 65°C.

30. (Currently Amended) The G1cNAc-phosphotransferase of Claim ~~26~~ 30, wherein said α -subunit comprises amino acids 1-928 of SEQ ID NO:4.

31. (Original) The G1cNAc-phosphotransferase of Claim 26, wherein said β subunit amino acids 1 to 328 of SEQ ID NO:5.

32. (Original) The GlcNAc-phosphotransferase of Claim 26, wherein said GlcNAc-phosphotransferase further comprises a γ subunit.

33. (Currently Amended) The GlcNAc-phosphotransferase of Claim 32, wherein said γ subunit is encoded by SEQ ID NO:6, or a nucleotide sequence that hybridizes under stringent conditions to the complement of SEQ ID NO:6, wherein said stringent conditions comprise hybridization in 50% formamide, 1M NaCl, 1% SDS at 37°C and washing in 0.1 X SSC at 60 to 65°C.

34. (Original) The GlcNAc-phosphotransferase of Claim 32, wherein said γ subunit comprises the amino acid sequence of SEQ ID NO:7.

35. (Original) The GlcNAc-phosphotransferase of Claim 26, wherein said site-specific proteolytic cleavage site is selected from the group consisting of a Furin proteolytic cleavage site, a Factor Xa proteolytic cleavage site, a Enterokinase proteolytic cleavage site, and a Genease I proteolytic cleavage site.

36. (Original) The GlcNAc-phosphotransferase of Claim 35, wherein said site-specific proteolytic cleavage site is a Furin proteolytic cleavage site.

37. (Currently Amended) The GlcNAc-phosphotransferase of Claim 36, wherein said Furin proteolytic cleavage site comprises SEQ ID NO:24 22.

Claims 38-53 (Cancelled).

54. (Withdrawn) A method of treating a patient suffering from a lysosomal storage disease comprising contacting a lysosomal hydrolase with the GlcNAc-phosphotransferase of Claim 26 to produce a lysosomal hydrolase with an N-acetylglucosamine-1-phosphate; removing said N-acetylglucosamine by contacting said lysosomal hydrolase with a phosphodiester α -GlcNAcase to produce a phosphorylated lysosomal hydrolase isolating said phosphorylated lysosomal hydrolase; and administering an amount sufficient to treat said disease the isolated phosphorylated lysosomal hydrolase.

55. (Withdrawn) A method of treating a patient suffering from a lysosomal storage disease comprising contacting a lysosomal hydrolase with the GlcNAc-phosphotransferase of Claim 22 32 to produce a lysosomal hydrolase with an N-acetylglucosamine-1-phosphate; removing said N-acetylglucosamine by contacting said lysosomal hydrolase with a phosphodiester α -GlcNAcase to produce a phosphorylated lysosomal hydrolase isolating said phosphorylated lysosomal hydrolase; and administering an amount sufficient to treat said disease the isolated phosphorylated lysosomal hydrolase.

56. (New) A method of phosphorylating a protein comprising contacting said protein with the isolated polypeptide of Claim 22 for a time and under conditions suitable to produce a phosphorylated protein.

57. (New) The method of Claim 56, wherein said protein comprises an asparagine-linked oligosaccharide with a high mannose structure.

58. (New) The method of Claim 56, wherein said soluble GlcNAc-phosphotransferase comprises an α subunit, a β subunit and a site-specific proteolytic cleavage site interposed between said α and β subunits, wherein said proteolytic cleavage site is not natural to said GlcNAc-phosphotransferase.

59. (New) The method of Claim 58, wherein said α subunit is encoded by nucleotides 165 to 2948 of SEQ ID NO:3, or a sequence that hybridizes under stringent conditions to the complement of nucleotides 165 to 2948 of SEQ ID NO:3, wherein said stringent conditions comprise hybridization in 50% formamide, 1M NaCl, 1% SDS at 37°C and washing in 0.1 X SSC at 60 to 65°C.

60. (New) The method of Claim 58, wherein said β -subunit is encoded by nucleotides 2949 to 3932 of SEQ ID NO:3, or a sequence that hybridizes under stringent conditions to the complement of nucleotides 2949 to 3932 of SEQ ID NO:3, wherein said stringent conditions

comprise hybridization in 50% formamide, 1M NaCl, 1% SDS at 37°C and washing in 0.1 X SSC at 60 to 65°C.

61. (New) The method of Claim 58, wherein said α -subunit comprises amino acids 1-928 of SEQ ID NO:4.

62. (New) The method of Claim 58, wherein said β subunit amino acids 1 to 328 of SEQ ID NO:5.

63. (New) The method of Claim 58, wherein said soluble G1cNAc-phosphotransferase further comprises a γ subunit.

64. (New) The method of Claim 63, wherein said γ subunit is encoded by SEQ ID NO:6, or a nucleotide sequence that hybridizes under stringent conditions to the complement of SEQ ID NO:6, wherein said stringent conditions comprise hybridization in 50% formamide, 1M NaCl, 1% SDS at 37°C and washing in 0.1 X SSC at 60 to 65°C.

65. (New) The method of Claim 63, wherein said γ subunit comprises the amino acid sequence of SEQ ID NO:7.

66. (New) The method of Claim 56, wherein said site-specific proteolytic cleavage site is selected from the group consisting of a Furin proteolytic cleavage site, a Factor Xa proteolytic cleavage site, a Enterokinase proteolytic cleavage site, and a Genease I proteolytic cleavage site.

67. (New) The method of Claim 66, wherein said site-specific proteolytic cleavage site is a Furin proteolytic cleavage site.

68. (New) The method of Claim 67, wherein said Furin proteolytic cleavage site comprises SEQ ID NO:24.

69. (New) The method of Claim 56, wherein said protein is a lysosomal hydrolase.

70. (New) The method of Claim 69, wherein said lysosomal enzyme is selected from the group consisting of α -glucosidase, α -iduronidase, β -galactosidase A, arylsulfatase, N-

acetylgalactosamine- α -sulfatase, β -galactosidase, iduronate 2-sulfatase, ceramidase, galactocerebrosidase, β -glucuronidase, Heparan N-sulfatase, N-Acetyl- α -glucosaminidase, Acetyl CoA- α -glucosaminide N-acetyl transferase, N-acetyl-glucosamine-6 sulfatase, Galactose 6-sulfatase, Arylsulfatase A, Arylsulfatase B, Arylsulfatase C, Arylsulfatase A Cerebroside, Ganglioside, Acid β -galactosidase G_{M1} Galglioside, Acid -galactosidase, Hexosaminidase A, Hexosaminidase B, α -fucosidase, α -N-Acetyl galactosaminidase, Glycoprotein Neuraminidase, Aspartylglucosamine amidase, Acid Lipase, Acid Ceramidase, Lysosomal Sphingomyelinase, Sphingomyelinase, and Glucocerebrosidase β -Glucosidase.

71. (New) The method of Claim 56, further comprising contacting said phosphorylated protein with an isolated phosphodiester α -GlcNAcase.

72. (New) The method of Claim 71, wherein said phosphodiester α -GlcNAcase comprises the amino acid sequence of SEQ ID NO:18.

73. (New) The method of Claim 71, wherein said phosphodiester α -GlcNAcase is encoded by a nucleotide sequence comprising SEQ ID NO:17 or a nucleotide sequence that hybridizes under stringent conditions to the complement of SEQ ID NO:17, wherein said stringent conditions comprise hybridization in 50% formamide, 1M NaCl, 1% SDS at 37°C and washing in 0.1 X SSC at 60 to 65°C.

74. (New) The method of Claim 56, wherein prior to said contacting the method comprises: culturing a host cell which comprises an isolated polynucleotide encoding soluble GlcNAc-phosphotransferase for a time under conditions suitable for expression of the soluble GlcNAc-phosphotransferase; and isolating said soluble GlcNAc-phosphotransferase.

75. (New) The method of Claim 56, wherein prior to said contacting the method comprises culturing a host cell which comprises an isolated polynucleotide encoding soluble GlcNAc-phosphotransferase for a time under conditions suitable for expression of the soluble GlcNAc-phosphotransferase, wherein said soluble GlcNAc-phosphotransferase

comprises an α subunit, a β subunit and a site-specific proteolytic cleavage site interposed between said α and β subunits, wherein said proteolytic cleavage site is not endogenous to G1cNAc-phosphotransferase; isolating said soluble G1cNAc-phosphotransferase; cleaving said isolated soluble G1cNAc-phosphotransferase with a proteolytic enzyme specific for said proteolytic cleavage site; and mixing said α and β subunits with a γ subunit of G1cNAc-phosphotransferase.

76. (New) A method of phosphorylating a protein comprising contacting said protein with the isolated polypeptide of Claim 26 for a time and under conditions suitable to produce a phosphorylated protein.

77. (New) The method of Claim 76, wherein said protein comprises an asparagine-linked oligosaccharide with a high mannose structure.

78. (New) The method of Claim 76, wherein said soluble G1cNAc-phosphotransferase comprises an α subunit, a β subunit and a site-specific proteolytic cleavage site interposed between said α and β subunits, wherein said proteolytic cleavage site is not natural to said G1cNAc-phosphotransferase.

79. (New) The method of Claim 78, wherein said α subunit is encoded by nucleotides 165 to 2948 of SEQ ID NO:3, or a sequence that hybridizes under stringent conditions to the complement of nucleotides 165 to 2948 of SEQ ID NO:3, wherein said stringent conditions comprise hybridization in 50% formamide, 1M NaCl, 1% SDS at 37°C and washing in 0.1 X SSC at 60 to 65°C.

80. (New) The method of Claim 78, wherein said β -subunit is encoded by nucleotides 2949 to 3932 of SEQ ID NO:3, or a sequence that hybridizes under stringent conditions to the complement of nucleotides 2949 to 3932 of SEQ ID NO:3, wherein said stringent conditions comprise hybridization in 50% formamide, 1M NaCl, 1% SDS at 37°C and washing in 0.1 X SSC at 60 to 65°C.

81. (New) The method of Claim 78, wherein said α -subunit comprises amino acids 1-928 of SEQ ID NO:4.

82. (New) The method of Claim 78, wherein said β subunit amino acids 1 to 328 of SEQ ID NO:5.

83. (New) The method of Claim 78, wherein said soluble GlcNAc-phosphotransferase further comprises a γ subunit.

84. (New) The method of Claim 83, wherein said γ subunit is encoded by SEQ ID NO:6, or a nucleotide sequence that hybridizes under stringent conditions to the complement of SEQ ID NO:6, wherein said stringent conditions comprise hybridization in 50% formamide, 1M NaCl, 1% SDS at 37°C and washing in 0.1 X SSC at 60 to 65°C.

85. (New) The method of Claim 83, wherein said γ subunit comprises the amino acid sequence of SEQ ID NO:7.

86. (New) The method of Claim 76, wherein said site-specific proteolytic cleavage site is selected from the group consisting of a Furin proteolytic cleavage site, a Factor Xa proteolytic cleavage site, a Enterokinase proteolytic cleavage site, and a Genease I proteolytic cleavage site.

87. (New) The method of Claim 86, wherein said site-specific proteolytic cleavage site is a Furin proteolytic cleavage site.

88. (New) The method of Claim 87, wherein said Furin proteolytic cleavage site comprises SEQ ID NO:24.

89. (New) The method of Claim 76, wherein said protein is a lysosomal hydrolase.

90. (New) The method of Claim 89, wherein said lysosomal enzyme is selected from the group consisting of α -glucosidase, α -iduronidase, β -galactosidase A, arylsulfatase, N-acetylgalactosamine- α -sulfatase, β -galactosidase, iduronate 2-sulfatase, ceramidase, galactocerebrosidase, β -glucuronidase, Heparan N-sulfatase, N-Acetyl- α -glucosaminidase,

Acetyl CoA- α -glucosaminide N-acetyl transferase, N-acetyl-glucosamine-6 sulfatase, Galactose 6-sulfatase, Arylsulfatase A, Arylsulfatase B, Arylsulfatase C, Arylsulfatase A Cerebroside, Ganglioside, Acid β -galactosidase G_{M1} Galglioside, Acid -galactosidase, Hexosaminidase A, Hexosaminidase B, α -fucosidase, α -N-Acetyl galactosaminidase, Glycoprotein Neuraminidase, Aspartylglucosamine amidase, Acid Lipase, Acid Ceramidase, Lysosomal Sphingomyelinase , Sphingomyelinase, and Glucocerebrosidase β -Glucosidase.

91. (New) The method of Claim 76, further comprising contacting said phosphorylated protein with an isolated phosphodiester α -GlcNAcase.

92. (New) The method of Claim 91, wherein said phosphodiester α -GlcNAcase comprises the amino acid sequence of SEQ ID NO:18.

93. (New) The method of Claim 91, wherein said phosphodiester α -GlcNAcase is encoded by a nucleotide sequence comprising SEQ ID NO:17 or a nucleotide sequence that hybridizes under stringent conditions to the complement of SEQ ID NO:17, wherein said stringent conditions comprise hybridization in 50% formamide, 1M NaCl, 1% SDS at 37°C and washing in 0.1 X SSC at 60 to 65°C.

94. (New) The method of Claim 96, wherein prior to said contacting the method comprises: culturing a host cell which comprises an isolated polynucleotide encoding soluble GlcNAc-phosphotransferase for a time under conditions suitable for expression of the soluble GlcNAc-phosphotransferase; and isolating said soluble GlcNAc-phosphotransferase.

95. (New) The method of Claim 76, wherein prior to said contacting the method comprises culturing a host cell which comprises an isolated polynucleotide encoding soluble GlcNAc-phosphotransferase for a time under conditions suitable for expression of the soluble GlcNAc-phosphotransferase, wherein said soluble GlcNAc-phosphotransferase comprises an α subunit, a β subunit and a site-specific proteolytic cleavage site interposed between said α and β subunits, wherein said proteolytic cleavage site is not endogenous to

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G1cNAc-phosphotransferase;isolating said soluble G1cNAc-phosphotransferase; cleaving said isolated soluble G1cNAc-phosphotransferase with a proteolytic enzyme specific for said proteolytic cleavage site; and mixing said α and β subunits with a γ subunit of G1cNAc-phosphotransferase.